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Differentiation of European cattle by AFLP fingerprinting

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Summary

The Neolithic introduction of domestic cattle into Europe was followed by differential adaptation, selection, migration and genetic isolation, leading ultimately to the emergence of specialized breeds. We have studied the differentiation of European cattle by amplified fragment length polymorphism (AFLP) fingerprinting. Combining AFLP data sets from two laboratories yielded 81 biallelic polymorphic markers scored in 19–22 individual animals from 51 breeds. Model-based clustering differentiated Podolian cattle as well as French and Alpine breeds from other European cattle. AFLP genetic distances correlated well with microsatellite-based genetic distances calculated for the same breeds. However, the AFLP data emphasized the divergence of taurine and indicine cattle relative to the variation among European breeds and indicated an Eastern influence on Italian and Hungarian Podolian breeds. This probably reflects import from the East after the original introduction of domestic cattle into Europe. Our data suggest that Italian cattle breeds are relatively diverse at the DNA sequence level.

Keywords amplified fragment length polymorphism, cattle, genetic diversity, introgression, zebu.

Introduction

Genetic differentiation of animal breeds has been compared at the DNA level using genetic markers. These studies have revealed the genetic complexity of the domestication process, migration routes and relationships among current breeds (Hanotte *et al.* 2002; Bruford *et al.* 2003; Cymbron *et al.* 2005; Beja-Pereira *et al.* 2006; Freeman *et al.* 2006). DNA analysis may elucidate the molecular background of the phenotypic variation among breeds and suggest priorities for conservation (Ruane 1999; Hall 2004).

Analyses of mitochondrial DNA and microsatellite loci have indicated that taurine and zebu cattle were domesticated independently (Bradley *et al.* 1996) and that many of the African and Middle-Eastern breeds are of mixed origin (Moazami-Goudarzi *et al.* 2001; Hanotte *et al.* 2002; Kumar *et al.* 2003; Freeman *et al.* 2004; Ibeagha-Awemu *et al.* 2004). Selective breeding and genetic isolation of taurine European cattle has resulted in many specialized dairy and beef breeds, several of which are now used worldwide. Allele frequencies of microsatellite markers reveal a genetic differentiation of breeds (e.g., see MacHugh *et al.* 1997; Moazami-Goudarzi *et al.* 1997; Peelman *et al.* 1998; Martín-Burriel *et al.* 1999; Schmid *et al.* 1999; Kantanen *et al.* 2000; Cañón *et al.* 2001; Del Bo *et al.* 2001; Wiener *et al.* 2004; Cymbron *et al.* 2005). However, the breeds in the data sets reported so far represent only partially the diversity of European cattle.

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Amplified fragment length polymorphism (AFLP) fingerprinting detects variation that corresponds to SNPs and indels and is informative for genetic diversity (Bensch & Åkesson 2005; Foulley *et al.* 2006; SanCristobal *et al.* 2006). Ajmone-Marsan *et al.* (2002) and Negrini *et al.* (2006) demonstrated the use of AFLP fingerprinting for estimation of genetic distances within and across cattle breeds. Here we analyze 47 European breeds, one African breed and three Indian zebu breeds in order to study the genetic differentiation of cattle across Europe.

Material and methods

Animals

DNA was isolated using standard procedures from blood or sperm samples. Collection of European (European Cattle Genetic Diversity Consortium 2006, Fig. 1), African (Moazami-Goudarzi *et al.* 2001) and Asian (Bradley *et al.* 1994; Loftus *et al.* 1994) cattle was described previously. Other breeds were sampled in Italy, including Holstein-Friesian and Limousin cattle. Microsatellite as well as AFLP genotypes

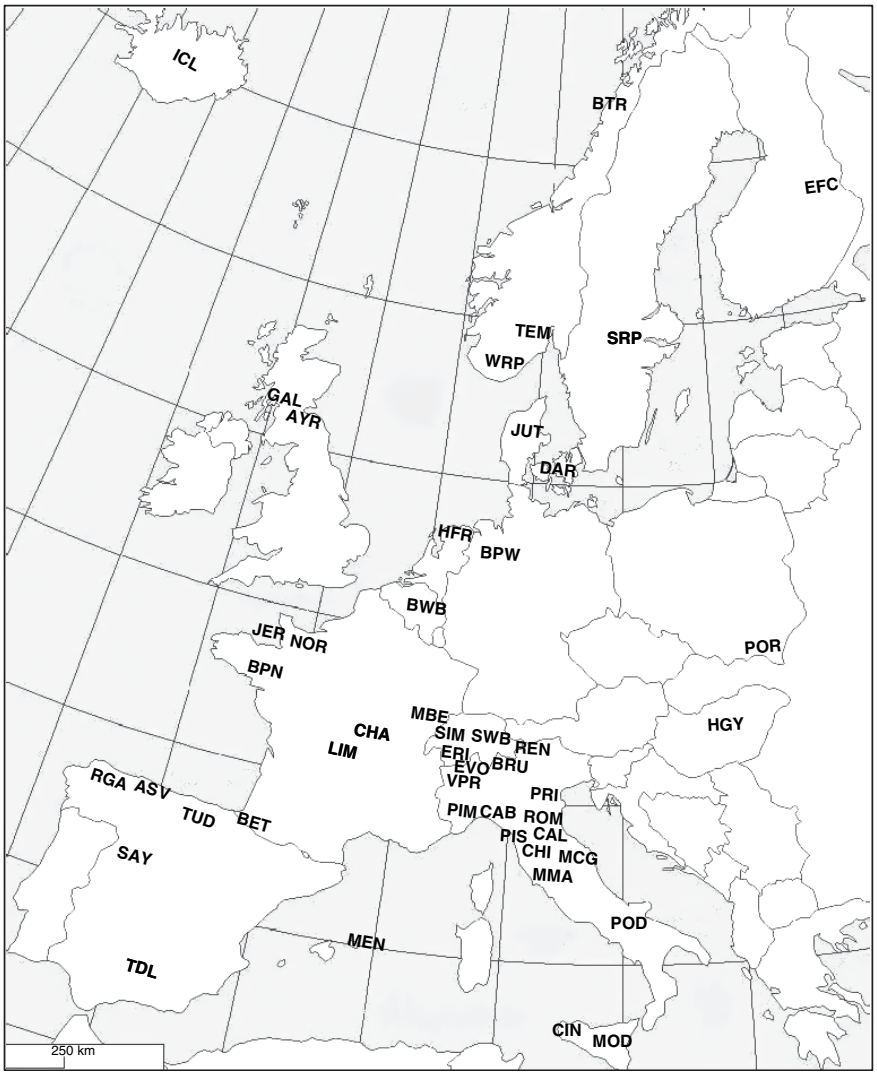

Figure 1 Origin of European breeds sampled in this study. ASV, Asturiana de los Valles; AYR, Ayrshire; BET, Betizu; BPW; German Black-Pied Western Reserve; BPN, Bretonne Pie Noire; BRU, Bruna Alpina; BTR, Blacksided Troender and Nordland; BWB, Belgian White-Blue; CAB, Cabannina; CAL, Calvana; CHA, Charolais; CHI, Chianina; CIN, Cinisara; DAR, Danish Red; EFC, Eastern Finn Cattle; ERI, Eringer; EVO, Evolenard; HFR, Holstein Friesian; GAL, Galloway; HGY, Hungarian Grey; ICL, Icelandic; JER, Jersey; JUT, Jutland; LIM, Limousin; MBE, Montbéliard; MCG, Marchigiana; MEN, Minorcan (Menorquina); MMA, Maremmana; MOD, Modicana; NOR, Normande; PIM, Piemontese; PIS, Mucca Pisana; POD, Podolica; POR, Polish Red; PRI, Italian Red Pied (Pezzata Rossa Italiana); REN, Rendena; RGA, Galician Blond (Rubia Gallega); ROM, Romagnola; SAY, Sayaguesa; SIM, Simmental; SRP, Swedish Red-Polled; SWB, Swiss Brown; TDL, Fighting Cattle (Toro de Lidia); TEM, Telemark; TUD, Tudanca; VPR, Aosta Red Pied (Valdostana Pezzata Rossa); WRP, Western Red Polled. AYR and HFR have been sampled outside their region of origin.

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indicated that 13 Betizu animals from the same location were similar to animals from two other locations, but highly inbred. As this confounded the clustering and distance plots, this inbred Betizu subpopulation was excluded from the analyses.

Molecular analysis

Genotyping of the AFLPs was carried out as described previously with the enzymes ECORI and TAQI and the primer combinations E35-T32, E39-T33 and E45-T32 (Ajmone-Marsan *et al.* 1997) using a commercial service (Keygene) or in the Piacenza Laboratory (Table S1). Genotyping of the 30 microsatellite loci recommended by the Food and Agriculture Organization for genetic diversity studies (http://dad.fao.org/en/refer/library/guidelin/marker.pdf) was carried out by commercial service (Labogena, France) or by the laboratories that carried out the sampling (Table S1). Additional genotypes of a subset of 19 microsatellite loci for a number of Asian and Italian breeds are from Loftus *et al.* (1999) and Cymbron *et al.* (2005).

Model-based clustering (Pritchard *et al.* 2000) was carried out using the STRUCTURE program (http://pritch.bsd.uchicago.edu/structure.html). As recommended in the program documentation for dominant genotypes, the no-admixture ancestry model was used, which yielded the best differentiation in combination with independent allele frequencies. Alternative models gave very similar results with only slightly reduced differentiation of clusters. For most runs, 20 000 burn-ins were followed by 50 000 iterations. This gave reproducible patterns, which were not improved by longer runs. The output of STRUCTURE was visualized by the DISTRUCT program (www-hto.usc.edu/~noahr/distruct.html).

F_{ST}values (Lynch & Milligan 1994) and Bayesian estimates of Nei's standard (D_s) and Reynolds (D_R) genetic distances between breeds or clusters of breeds were calculated on the basis of non-uniform prior distribution of allele frequencies using the AFLPsurv program (Vekemans 2002, http://www.ulb.ac.be/sciences/lagev/aflp-surv.html). Neighbor-Net graphs (Bryant & Moulton 2004) based on D_s distances were constructed with the SplitsTree program (http://www.splitstree.org). Average values of the Jaccard band-sharing index were calculated with the program POPDIST (A. Valentini, Viterbo).

Results

Genotyping and combination of data sets

Three enzyme-primer combinations were selected that optimally displayed polymorphisms in the cattle genome (Ajmone-Marsan *et al.* 1997). Analysis in the Keygene and Piacenza laboratories gave 115 and 143 polymorphic AFLP fragments respectively. The size range that could be scored in both laboratories contained 81 unambiguous polymorphic bands and 163 monomorphic bands.

The correspondence between data sets was tested by typing five breeds in both laboratories and by independent sampling and fingerprinting of French and Italian populations of Limousin cattle. An error rate of 2% in the 81 common markers was found for 162 Italian samples genotyped in duplicate, which is considered typical for AFLP-based population genetic studies (Bonin *et al.* 2004; Gorni *et al.* 2004). Genetic distances between European taurine breeds were in the range of 0.008–0.040, but were 0.00005 or less for the same breed analyzed in two laboratories and 0.0035 between the French and Italian Limousin populations.

Twenty-two randomly selected animals per breed were included in the analysis by model-based clustering in order to avoid a bias towards over-represented breeds.

Genetic subdivision

Values for the fixation index (F_{ST}) on the basis of dominant data (Lynch & Milligan 1994) were estimated to be 0.15 for

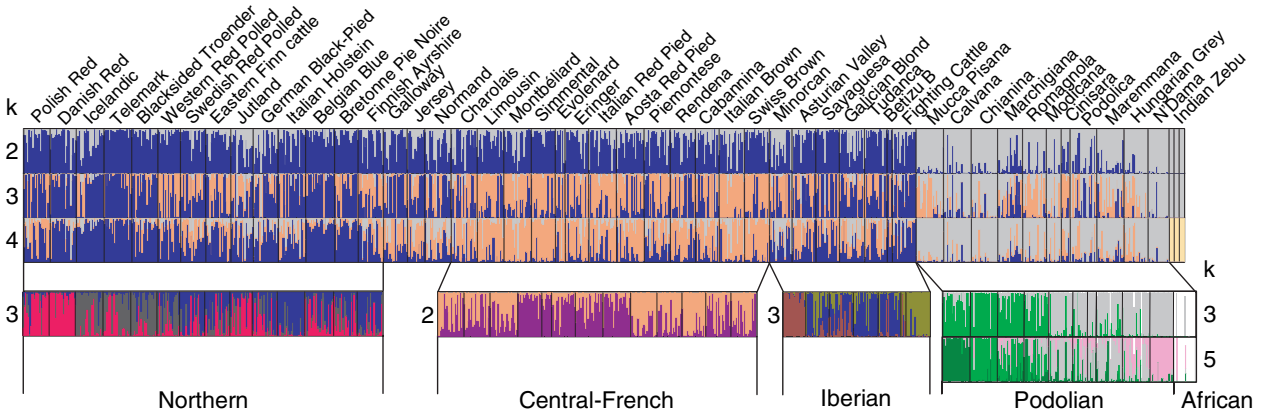

Figure 2 Model-based clustering of AFLP fingerprints from all 51 breeds or from regional subsets of breeds. Individuals are represented by lines, the colors of which indicate the likelihood of belonging to one of the *k* inferred clusters.

the complete data set, 0.099 within Europe and slightly lower (0.089) within Europe excluding the Podolian breeds. Genetic subdivision was further analyzed by model-based clustering (Pritchard *et al.* 2000) testing several numbers of clusters (k).

Analysis at $k = 2$ separated Indian zebu, African N'Dama, Italian and Hungarian Podolian cattle from other European breeds (Fig. 2). Incomplete differentiation at $k = 3$ and $k = 4$ suggested a separate cluster of French and Alpine breeds with intermediate positions for Jersey, Galloway and Normande. Indian zebu breeds were differentiated from African and Podolian cattle at $k = 4$. In a data set in which the number of European and African animals was reduced to 62 individuals (data not shown), the first split at $k = 2$ was between zebu and taurine, followed at $k = 3$ by a split between European and African animals. This indicates that the clustering inferred at $k = 2$ and $k = 3$ in the complete data set was influenced by the under-representation of Indian and African cattle.

Although the likelihood of the data increased steadily with k-values from 2 to 10, k values higher than 4 did not detect additional clusters of breeds (not shown). However, a further subdivision was suggested by analyzing separately the breeds from different regions (Fig. 2). Within the Northern breeds, Danish and Polish Red cattle as well as Nordic cattle tended to form separate clusters. Likewise, a cluster within the French-Alpine group is formed by the spotted Simmental-like cattle (Fleckvieh breeds) together with the Southern Swiss Evolenard and Eringer, but excluding the French Montbéliard. In Iberian cattle, the inbred Minorcan breed and fighting bulls were separated from other breeds. Analysis of Podolian and African breeds generated clusters for African cattle, the Pisana, the Chianina with the closely related Calvana and the Hungarian Grey respectively.

Genetic distances

We choose the Nei standard genetic distance D_s because of its linearity with divergence time (Laval *et al.* 2002). Comparison of D_s values with the average across-breed values of the Jaccard index of AFLP band sharing of individuals showed a good correlation (Fig. 3a), indicating that D_s also measures the sequence divergence between breeds. In contrast, Reynolds distances, which are recommended as measure for the divergence of closely related populations (Laval *et al.* 2002), were only colinear with the Jaccard values for taurine breeds.

As shown in Fig. 3b for taurine breeds, the D_s values were five to seven times higher than D_s values calculated from microsatellite data (European Cattle Genetic Diversity Consortium 2006). However, genotypes for 19 microsatellite markers from zebu breeds (MacHugh *et al.* 1997; Freeman *et al.* 2006) a lower ratio of microsatellite- and ALFP- based D_s distances in a comparison of taurine to zebu breeds (4.2–4.5, Fig. 3c), presumably because of a saturation of the microsatellite-based distance after longer periods of divergence.

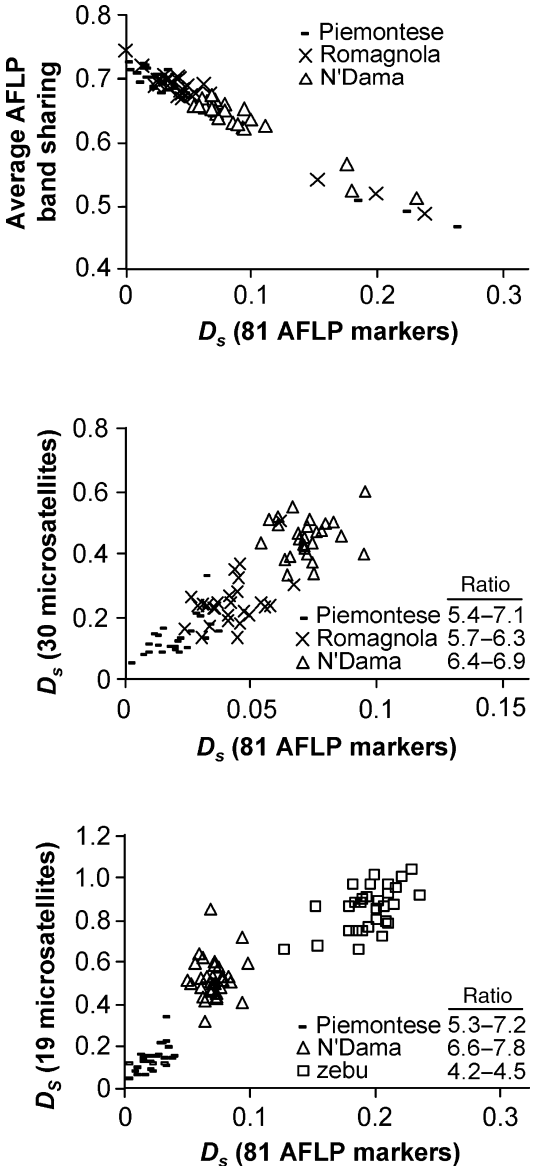

Figure 3 Genetic distances between breeds derived from ALFP data. (a) Nei's standard distance D_s between the indicated breed and other breeds against average across-breed Jaccard index of band sharing. Monomorphic bands have not been taken into account. (b) D_s distances between the indicated breeds and other breeds against the corresponding distance on the basis of 30 microsatellites. (c) D_s distances between the indicated taurine breeds and zebu respectively and all other breeds plotted against the corresponding distance on the basis of 19 microsatellites. The range of ratios of the AFLP- and microsatellite-based distances indicates the central 90% confidence interval as calculated by linear regression.

This difference between markers was also apparent from a visualization of the D_s distances of regional groups of breeds in NeighborNet graphs (Fig. 4). These graphs visualize conflicting tree topologies that may correspond to reticulated relationships of interacting populations (Bryant &

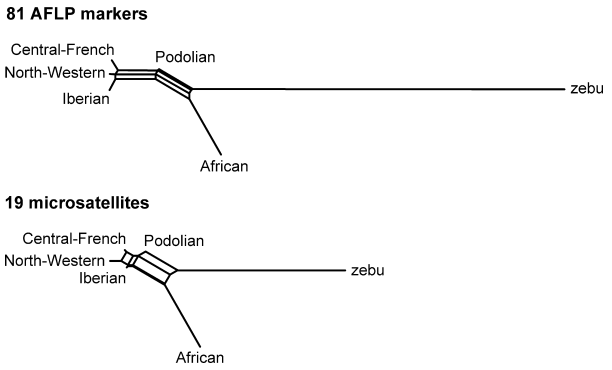

Figure 4 NeighborNet graphs of D_s distances for groups of breeds analysed by AFLP fingerprinting or by typing with 19 microsatellites (see Table S1).

Moulton 2004). Podolian cattle were positioned between the African N'Dama and the zebus on the basis of AFLP data, while microsatellite-based distances clustered all European cattle together.

Discussion

In an earlier study, we showed that AFLPs can be used to compare related bovine species (Buntjer *et al.* 2002) and bovine breeds (Ajmone-Marsan *et al.* 2002; Negrini *et al.* 2006). The results of the present study indicate that for assessing genetic diversity, AFLP markers contribute information that is complementary to microsatellite marker data (*cf.* Gaudeul *et al.* 2004; Foulley *et al.* 2006; SanCristobal *et al.* 2006). In a separate study (unpublished results), we found that model-based clustering of microsatellite genotypes differentiated several cattle breeds or breed groups from northern and western Europe at lower *k*-values than Mediterranean breed groups. In contrast, the AFLP data set used here discriminated Podolian from other European cattle at $k = 2$ and only partially differentiated northern and western breeds. Genetic distances between taurine and indicine breeds based on microsatellite allele frequencies were relatively short (Fig. 3c, Fig. 4). These observations indicate that, relative to microsatellite variation, AFLP emphasizes the differentiation between zebu and taurine cattle and between taurine breeds from Eastern and European origin. Because most variation in AFLP fingerprints corresponds to SNPs or indels, our study predicts that a European-wide SNP analysis will highlight the Eastern component in Podolian cattle and the separate position of the Alpine-French breeds.

Principal coordinate analysis of European breeds (results not shown) reproduced only the separation of Podolian and non-Podolian European breeds. Model-based clustering (Pritchard *et al.* 2000) reconstructed subdivisions based on individual genotypes and potentially detected clusters of related breeds or introgression events. This approach has been used for clustering of microsatellite genotypes of chicken (Rosenberg *et al.* 2001) and goat (Cañón *et al.* 2006) breeds and to simulated AFLP data (Evanno *et al.* 2005). One caveat is that the inferred clusters depend not only on the divergence of populations, but also on the composition of the data set. In this study, zebu breeds formed a separate cluster only at $k = 4$ because of their numerical under-representation. Conversely, inclusion in the data set of 45 additional Maremmana animals led to the identification of a separate cluster for this breed.

Although the differentiation of the non-Podolian European breeds with the current data set was incomplete, the suggested clusters correlated with the geographical origin: the Danish and Polish Red cattle from the Baltic region, Nordic cattle, a larger group of breeds from France and the Alpine regions, and within this group a cluster of Simmental-like breeds and two Southern Swiss breeds. Within Iberian and Podolic cattle, separate clusters were defined for the inbred Minorcan, Fighting cattle and Pisana (derived from a cross of Chianina and Swiss Brown) breeds and for the Hungarian Grey cattle.

The clustering of the Alpine and French breeds indicates a shared history, possibly the legacy of genetic bottlenecks in the Alpine valleys during the spreading of domestic cattle to the West.

Model-based clustering as well as networks based on genetic distances indicated an influence of Eastern cattle in the Italian and Hungarian Podolian breeds, confirming earlier results based on biochemical data (Baker & Manwell 1980; Medjugorac *et al.* 1994; Pieragostini *et al.* 2000). Because it is unlikely that there were frequent contacts between European taurine and indicine or other Eastern cattle, the most realistic scenario is a gene flow via Middle-Eastern and Balkan breeds (Beja-Pereira *et al.* 2006). Grey steppe breeds have been kept from the 12th century on the plains north-west of the Black Sea (Felius 1995) and have presumably a more Eastern origin. However, Chianina cattle are supposed to descend from large white cattle already described in texts from the Roman era (Negrini *et al.* 2006). Mitochondrial haplotypes also suggest introgression of aurochs in Italian cattle (Beja-Pereira *et al.* 2006). Middle-Eastern cattle may have been brought to imperial Rome as tributes (Pieragostini *et al.* 2000), but probably not in substantial numbers. Later introgression may have occurred during migrations or invasions of the Visigoths and Huns (Alderson 1992; Felius 1995) and by trade in the 14th and 15th centuries (Felius 1995). Thus several historic events and processes may have contributed to the high genetic diversity of Italian cattle, which also harbors several distinct Alpine breeds and the composite Piemontese (Felius 1995).

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Appendix 1.

The following members of the European Cattle Genetic Diversity Consortium contributed to this work:

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Supplementary material

The following supplementary material is available for this article online from http://www.blackwell-synergy.com/doi/full/10.1111/j.1365-2052.2007.01554.x

Table S1 Breeds analyzed by AFLP fingerprinting.

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